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Lipid Raft and Platelet SNARE Machinery

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Lipid rafts are defined as insoluble areas in the cell membrane, resistant to non-ionic detergents. These areas, which are also called detergent-resistant membranes (DRMs), and are enriched in glycosphingolipids, saturated phospholipids and cholesterol, have been identified in several cell types including platelets. Initially, they were believed to be responsible for the intercellular transport of glycosyl phosphatidylinositol (GPI)-anchored proteins to the apical surface in polarized cells [1,2]. However, over the decades, rafts have increasingly been recognized as membrane microdomains, and found to play a critical role in the control of several cellular activation processes. Thus, very divergent proteins such as Src family kinases, caveolins, palmitoylated proteins such as G proteins, GPI-anchored proteins such as Thy-1 and alkaline phosphatases, tetraspannin proteolipids and various signaling molecules have all been shown to be associated with lipid rafts. Different types of rafts coexist at the plasma membrane with functionally distinct lipid composition [3]. Furthermore, lipid rafts are not only found at the plasma membrane, but also as part of the internal membrane of granules, Golgi complex and even phagosomes [4,5]. Evidence for a functional role of lipid rafts in platelets is very recent: Gousset et al. [6] have shown that during cold-induced platelet activation, rafts cluster into larger aggregates, a reversible process depending on platelet activation. These authors showed raft aggregation to be dependent on the presence of cholesterol in the membrane, and further identified the presence of CD36 in DRMs. Using fluorescence microscopy of platelets being activated with thrombin and collagen, large fluorescent clusters of lipid rafts were formed, leading these investigators to conclude that raft aggregation is triggered by platelet activation, suggestive of a role for microdomains in platelet signaling [6].

An important physiological consequence of human platelets signaling is exocytosis, a process that involves secretory granule fusion with the platelet plasma membrane. This membrane fusion reaction is mediated by membrane proteins called Soluble *N*-ethylmaleimide sensitive factor attachment protein receptors (SNAREs). In platelets, vesicle-associated membrane protein (VAMP)-8/endobrevin (a vesicle, or v-SNARE) is required for each granule type [7]. Two target membrane SNAREs (*t*-SNAREs) are present: the syntaxin class (syntaxin-11 being functionally relevant) [8] and the SNAP-23/25 class (SNAP-23 being functionally relevant) [9-14]. Either immediately preceding or concurrent with membrane fusion, three cognate SNARE proteins on opposing membranes assemble, through hydrophobic interactions in their coiled-coil domains, into a parallel, four helical bundle [15-17]. This so-called *trans* complex, containing one copy each of SNAP-23/syntaxin/VAMP, is minimally required for membrane fusion [18] and on fusion, the SNAREs exist in the same membrane in a thermodynamically stable *cis* configuration [19]. This *cis* SNARE complex has to be disassembled by the adapter proteins N-ethylmaleimide-sensitive fusion protein and α-SNAP in a process called SNARE priming [20,21], thereby allowing SNAREs to be reused in another round of membrane fusion. However, what the function of NSF and α-SNAP is in platelets is still unknown, and how the SNARE recycling takes place in platelets is also unknown. The SNAREs form a *trans*-bilayer complex, which facilitates fusion of the granule and plasma membranes for cargo release. Although SNAREs are essential for fusion, how, when, and where they assemble into fusogenic complexes represent key secretion control points. Thus, SNARE complex assembly is determined by numerous SNARE regulators (i.e., Munc18s and Munc13s) and post-translational modifications (i.e., acylation and phosphorylation) [15].

The SNARE proteins have also been reported to associate with lipid rafts in other secretory cells [22,23]. SNAP-23 and SNAP-25 associate with membranes by palmitoylation [24-28], and it is likely that this post-translational modification is responsible for the association of these proteins with lipid rafts. By contrast, members of the syntaxin and synaptobrevin/VAMP family are generally not palmitoylated, and their mechanism of association with rafts remains unknown. In addition, it is unclear to what extent individual SNARE subunits or binary/ternary SNARE complexes associate with lipid raft membranes or whether the nature of SNARE complexes associated with rafts is altered during the process of regulated exocytosis.

Karim et al. [8] have been examining the membrane distribution of individual SNARE proteins and SNARE complexes that regulate granule exocytosis from human platelets (unpublished). The role of lipid rafts in platelet exocytosis is still unclear. Nonetheless, it is interesting that SNAP-23 appears to localize to rafts yet for biophysical reasons, rafts appear to be an unlikely site for membrane fusion events given the rigidity of their structure. It seems possible that phosphorylation could be an exclusion signal that prevents a portion of the acylated SNAP-23 from associating with the rafts.

Finally, it is very important to notice that cholesterol is at the core of the platelet lipid-raft function. Could cholesterol-lowering drugs directly affect platelet function, and could their action be via an effect on the platelet rafts? Certainly statins have been shown to reduce platelet-dependent thrombus formation [29], and such effects could explain the rapid therapeutic benefits of statin therapy [30]. Much further research lipid rafts is warranted and also it is important to understand how lipid rafts are involved in platelet exocytosis.

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